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# Denitrifying microbial communities along a boreal stream with varying land-use

Sanni L. Aalto<sup>1,2</sup> · Jatta Saarenheimo<sup>2</sup> · Lauri Arvola<sup>3</sup> · Marja Tiirola<sup>2,4</sup> · Jussi Huotari<sup>3</sup> · Antti J. Rissanen<sup>5</sup>

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## Abstract

Streams have an important role in regulating nitrogen (N) transportation from terrestrial ecosystems to downstream waters. Here, we examined how catchment land-use affects potential denitrification rates and the function and composition of denitrifier communities in boreal stream sediments, using stable isotope incubations and qPCR and 454-pyrosequencing targeted on *nirS*, *nirK* and *nosZ* genes. Although land-use influenced the water chemistry as higher nitrite + nitrate ( $\text{NO}_x^-$ ) concentration at the agriculture-affected sampling point, sediment organic matter content was found to be the key factor in regulating potential denitrification rates. However, the abundance as well as the diversity and community composition of denitrifying microbes, and genetic  $\text{N}_2\text{O}$  production potential (the ratio between *nirS* + *nirK* and *nosZ* gene abundances) were connected to both  $\text{NO}_x^-$  and sediment quality. Overall, our results suggest that catchment land-use-driven changes in N and carbon availability affect the denitrification rates, and possibly  $\text{N}_2$ : $\text{N}_2\text{O}$  production ratio, in boreal streams, through altering denitrifier abundance and community composition.

**Keywords** Catchment land-use · Community composition · Denitrification · *nirS* + *nirK* · *nosZ* · 454-Pyrosequencing

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00027-019-0654-z>) contains supplementary material, which is available to authorized users.

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## Introduction

Anthropogenic activities such as application of nitrogen (N) fertilizers and waste disposals have substantially increased N loadings into aquatic ecosystems (e.g. rivers, lakes; Galloway et al. 2014). Streams have an important role in regulating N transport, acting as entries for the terrestrial material to the hydrological continuums of rivers, lakes and oceans. Even small streams may have relatively high inorganic nitrogen removal capacity, and can retain substantial amounts of biologically reactive inorganic N species, ammonium and nitrate, through assimilation to the biotic material, sedimentation or denitrification (Peterson et al. 2001; Jarvie et al. 2018). Denitrification is a microbial process, where inorganic N (nitrate) can be reduced to biologically inert nitrogen gas ( $\text{N}_2$ ), which is then released from the ecosystem, making it the only process truly removing N from the systems. Denitrification is a sequential process with four enzymatic steps. Genes coding for enzymes reducing nitrite ( $\text{NO}_2^-$ ) to nitric oxide (NO), *nirS* and *nirK*, are the most widely used molecular markers to study denitrifier populations. Besides nitrite reductases, nitrous oxide ( $\text{N}_2\text{O}$ ) reductase (coded by *nosZ*) has an important role in controlling the final step of denitrification, and the high ratio between the

abundances of *nir* and *nos* genes has found to be an indicator for the high potential production of greenhouse gas  $N_2O$  (Saarenheimo et al. 2015a).

In streams, denitrification rates are expected to increase with nitrate concentrations (Seitzinger et al. 2006), although oxygen concentration and temperature may be more important factors in driving seasonal variations in stream denitrification rates (Inwood et al. 2005; Jarvie et al. 2018). Higher phosphorus availability has been suggested to promote in-stream denitrification as well (Eberhard et al. 2018). Furthermore, the proportion of removed N has been found to decrease in agricultural streams with high N concentrations, which is mainly explained as organic matter availability limiting the process (Inwood et al. 2005, 2007; Arango et al. 2007; Mulholland et al. 2008).

Boreal aquatic ecosystems exhibit lower N removal rates than the ones in the temperate area (Rissanen et al. 2013), suggesting that a higher proportion of catchment N loading may be exported through stream–river–lake–continuum, and eventually discharged to vulnerable areas, such as the Baltic Sea. However, increasing anthropogenic N loading can promote denitrifying communities and denitrification in the boreal water bodies (Bruesewitz et al. 2011; Aalto et al. 2018). Here, we examined the genetic potential and composition of sediment denitrifying microbial communities along

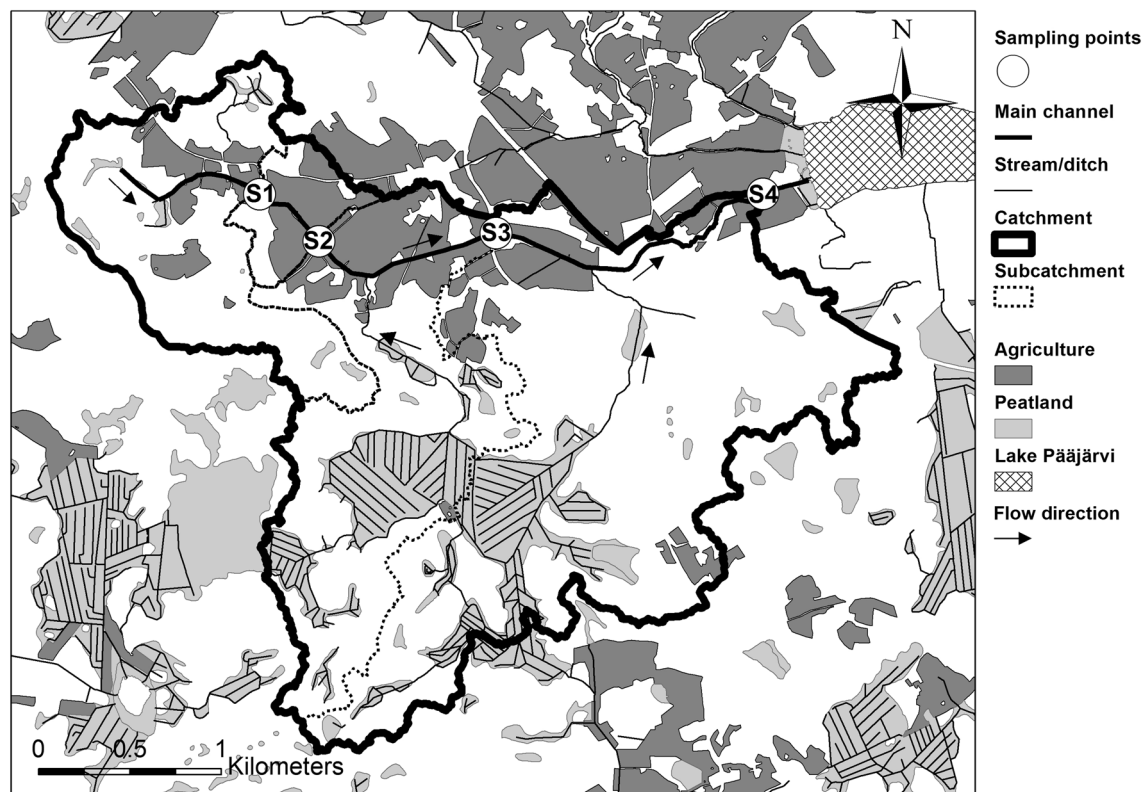
a gradient of different land-use types in a boreal stream, using quantitative polymerase chain reaction (qPCR) and 454-pyrosequencing targeted at *nirS*, *nirK* and *nosZ* genes. In addition, we measured sediment denitrification rates with isotope pairing technique (IPT) method (Nielsen 1992). We hypothesized that the stream microbial habitat conditions, reflecting the catchment land-use, shape the composition and function of denitrifying microbes and rates of denitrification.

## Materials and methods

### Sample and data collection

Löytynoja is a first-order stream (~4.5 km) in Southern Finland, which originates from a groundwater spring and discharges into the oligo-mesotrophic Lake Pääjärvi. Sampling was conducted along the stream at four points between 22 and 29 July 2010 (Fig. 1; Table 1). The sampling points differ in their catchment area and land-use (Table 1).

Sampling point S1 is located to the upper reaches of the stream with forest-dominated catchment area, point S2 is agriculture-dominated and receives high amounts of agricultural runoff. Sampling points S3 and S4 are located in the lower part of the stream, where the influence of drained



**Fig. 1** Map of stream Löytynoja catchment area. The white areas represent forest or built areas

**Table 1** The environmental variables in water above the sediment surface (oxygen: O<sub>2</sub>, temperature: temp, pH, DOC: dissolved organic carbon, NO<sub>x</sub><sup>-</sup>: nitrite and nitrate, PO<sub>4</sub><sup>-</sup>: phosphate, NH<sub>4</sub><sup>+</sup>: ammonium, TN: total nitrogen, TP: total phosphorus), and of the sediment

(LOI%: the proportion of organic matter, %C: sediment carbon content, %N: sediment nitrogen content), catchment area (land area), the proportions of each land-use type, and the denitrification potential (D15) at four sampling points

Sampling point	Sampling date	O <sub>2</sub> %	Temp	pH	DOC (mg/l)	NO <sub>x</sub> <sup>-</sup> (μmol/l)	PO <sub>4</sub> <sup>-</sup> (μmol/l)	NH <sub>4</sub> <sup>+</sup> (μmol/l)	TN (μmol/l)	TP (μmol/l)
S1	22 July 10	90.6	9.3	7.0	1.8	55.4	0.06	0.64	57.9	0.23
S2	28 July 10	93.5	8.8	6.9	1.8	74.8	0.23	0.71	83.2	0.68
S3	29 July 10	94.7	10.5	6.9	2.3	61.4	0.06	3.14	70.5	0.68
S4	23 July 10	95.5	12	7.2	2.8	48.5	0.10	1.93	52.3	0.58
	LOI%	%C	%N		Land area (ha)	Field (%)	Peatland (%)	Forest (%)		D15 (μmol N/m <sup>2</sup> /day)
S1	4.50	0.73	0.05		14,928	13.4	2.9	83.7		36.9
S2	6.25	2.95	0.29		3266	62.9	0	37.1		423.5
S3	3.05	0.99	0.05		22,083	18.1	23.8	58.0		16.1
S4	5.18	2.26	0.18		41,107	7.8	15.3	76.9		456.4

peatlands is higher than at the two other points. At S4, located near Pääjärvi where stream water is eventually discharged, there is a gauging station with spillway weir decreasing the water velocity (Fig. 1). More information of the stream can be found from Hakala et al. (2002), Huotari et al. (2013), and Arvola et al. (2015). The catchment area for each sampling point was delineated from digital elevation model (pixel size 2 m) based on a laser scanning (National Land Survey of Finland 2016), and the proportions of different land-use types (i.e. forest, agriculture, and peatland; Table 1) were defined using Topographic Database of National Land Survey of Finland (2010) and analysis with ArcMap 10. The catchment areas of the sampling points represent only the additional land area following the previous upstream sampling points and do not overlap. This approach was used since we wanted to focus only on the nearest catchment area, where most of the runoff is coming to the sampling point, rather than on the entire upper stream catchment area.

Four sediment cores (diameter of 7 cm) were collected from each sampling point using plexiglass tubes. Water temperature, oxygen concentration, and pH were measured above the sediment surface at each sampling point. Water carbon and nutrient content were analysed using Lachat's Flow Injection Analysis System (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, TN, and TP) and Shimadzu TOC 5000 analyzer (DOC) with standard methods (Arvola et al. 2014). Sediment porosity and organic matter content (LOI%, loss on ignition) were measured as previously described (Rissanen et al. 2011), and sediment %N and %C were measured from freeze-dried samples using Carlo-Erba Flash 1112 series Elemental Analyser (Thermo Scientific, Waltham, MA, USA). For DNA extractions, four subsamples were taken from the sediment surface layer (depth of 0–1 cm) and four

subsamples were taken from the lower sediment layers (deep sample from depths 1–5 cm). Samples were slurried, divided into 200 μl aliquots, and stored at –20 °C, as in Rissanen et al. (2011).

### Sediment denitrification rates

For measuring potential (D15) and actual (D14) denitrification rates, three to four subcores were taken using small plastic tubes (length 16 cm, diameter 2.6 cm) from each sediment core as in Rissanen et al. (2011). Shortly, the tubes were pushed into the sediment so that approximately 4–7 cm of the tube length was filled with sediment, and the rest with overlying water. Following IPT (isotope pairing technique; Nielsen 1992), subcores were incubated with <sup>15</sup>NO<sub>3</sub><sup>-</sup>-label (20, 70, 120 μM) with a magnetic stirrer on the lid of the cores, at in situ temperature and darkness. After 3 h, microbial activity was terminated by adding 1 ml ZnCl<sub>2</sub> (1 g/ml), subcores were mixed, and subsamples of the sediment–water slurry were transferred to gas-tight glass vials (12 ml; Exetainer®; Labco). The N<sub>2</sub> isotopic composition was measured with an isotope ratio mass spectrometer (Roboprep-G+ and Tracermass, Europa Scientific) at the National Environmental Research Institute in Silkeborg, Denmark.

### qPCR of *nirS*, *nirK*, and *nosZ*

Nucleic acids were extracted from sediment samples using a modified version of the bead-beating and phenol–chloroform extraction protocol of Griffiths et al. (2000) (see Rissanen et al. (2010) for further details). The abundance of *nirS*, *nirK*, and *nosZ* genes were measured with quantitative PCR (qPCR) as in Saarenheimo et al. (2015b). Before qPCR,

the four subsamples samples from each sampling depth and sampling point were pooled, resulting in eight samples in total. We used nirSCd3aF/nirSR3cd (Kandeler et al. 2006) primer pair for *nirS*, nirK876/nirK1040 (Henry et al. 2004) for *nirK*, and nosZ2F/nosZ2R (Henry et al. 2006) for *nosZ*. Annealing temperatures were 55 °C for *nirS*, and 60 °C for *nirK* and *nosZ*. No template inhibition was observed, when qPCR linearity was tested from original, 10- and 100-fold template dilution series (Saarenheimo et al. 2015b).

## 454 Pyrosequencing

The community structure, richness and diversity of organisms harbouring *nirS*, *nirK* and *nosZ* genes were studied using 454-pyrosequencing. PCR was conducted with primer pairs nirScd3aF/nirSR3cd for *nirS* (Kandeler et al. 2006), F1aCu/R3Cu for *nirK* (Hallin and Lindgren 1999), and nosZF/nosZ1622R for *nosZ* (Kloos et al. 2001; Throbäck et al. 2004) from four replicate sediment samples following Saarenheimo et al. (2015b). The sequencing and sequence data analysis were conducted as in Saarenheimo et al. (2015b) using samples pooled for sampling point and depth ( $n=8$ ). Sequences were subsampled (610 for *nirK*, 652 for *nirS*, and 312 for *nosZ*), classified into operational taxonomical units (OTUs) at the 90% similarity level, and community diversity (Shannon index; estimator of species richness and species evenness), species richness (Chao1 richness estimate; abundance-based estimator of species richness; Chao 1984), and a coverage estimate of the proportion of amplified gene amplicons represented by sequence libraries for each sample (Good's coverage) were calculated in Mothur (Schloss et al. 2009). Nucleotide sequences were assigned to taxonomies in Mothur following Saarenheimo et al. (2015b). The 454-pyrosequencing data were deposited to the NCBI's Sequence Read Archive under accession number SRP150895.

## Statistical analysis

All statistical analyses were conducted using R version 3.3.3 (R Core Team, 2017). Using the relative abundances of the most dominant OTUs (OTUs comprising > 80% of sequences across all samples), samples were UPGMA clustered, and heatmaps were created using function "pheatmap" in R. The relationships between environmental variables, land-use types, and gene abundances and their ratios, or diversity and species richness of the communities were studied with Spearman correlation.

## Results and discussion

### Environmental variables and denitrification rates

In Löytynoja, nutrient and carbon concentrations changed along the hydrological pathway from upstream to downstream due to the land-use of the sampling points (Table 1). Dissolved organic carbon as well as ammonium increased from the forest-dominated S1 to the S3 and S4, where peatland contributed more to the water quality, agreeing with the previous findings on the higher proportion of peatland in the catchment increasing DOC concentrations (Huotari et al. 2013; Laine et al. 2014), and on high ammonium loading coming from drained peatlands (Laine et al. 2013; Arvola et al. 2015). Nitrite + nitrate, phosphate, TN and TP peaked at agriculture-dominated S2, as observed previously in Löytynoja and in other agriculture-affected streams (Hakala et al. 2002; Inwood et al. 2005). In contrast to water chemistry, sediment quality (%C, %N, and LOI%) varied less consistently, being high both at S2 and S4, although these points had a different land-use coverage. At S2, agricultural loading with high organic matter content explains the result (Inwood et al. 2007). At S4, this result can be explained with the dam decreasing water velocity and leading to accumulation of organic matter coming from the upper catchment area and also from the previous sampling points. It seems that sediment quality was partly explained with land-use and partly with streambed characteristics and water velocity.

In IPT samples, the D15 did not increase in parallel with  $^{15}\text{N}$ -labeled nitrate concentration (Suppl. Figure 1), hence, the assumption of nitrate- limitation of denitrification in the IPT was not met (Nielsen 1992). Therefore, it was possible to calculate only potential denitrification as the average of D15 measured from cores receiving 120  $\mu\text{mol/l}$   $^{15}\text{NO}_3$  addition. We found that S2 and S4 were denitrification hotspots, the denitrification potential (D15) being 12–28 fold higher there as compared to S1 and S3 (Table 1). According to the correlation analysis, D15 increased with sediment quality (%C:  $\rho=0.94$ ,  $p<0.001$ , %N:  $\rho=0.87$ ,  $p<0.001$ , LOI:  $\rho=0.78$ ,  $p<0.001$ ), and with the stream water phosphate, but was not connected to nitrite + nitrate ( $p>0.05$ ; Suppl. Figure 2). In this environment, denitrification was expected to be mainly heterotrophic, so our result indicates that nitrate levels were always adequate, but carbon availability was regulating denitrification potential, explaining the lower denitrification potential found at S1 and S3 with low sediment C content. Previously, denitrification has found to be limited with carbon, or co-limited with nitrate and carbon in nitrate-rich streams (Arango et al. 2007; Inwood et al. 2007), and recently, higher nitrate uptake fluxes (Tomasek



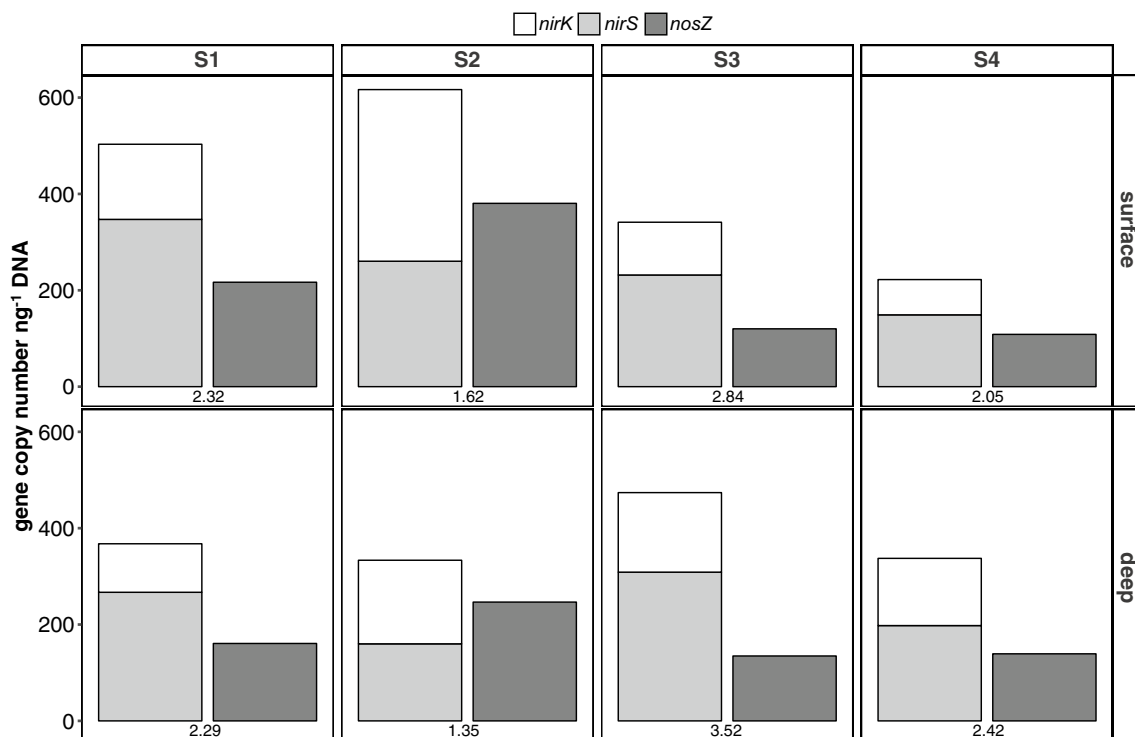
et al. 2018) and denitrification rates (Eberhard et al. 2018) were found in carbon-amended than in unamended stream sediments. Together with these previous findings, our results highlight the key role of carbon in supporting beneficial nitrate reduction in stream environments.

### Genetic denitrification potential

The abundance patterns of the three denitrification genes were not uniform (Fig. 2). There were some differences in the gene abundances between the surface and deeper sediment samples between the sampling points, as deeper samples had higher genetic denitrification potential than surface samples at S3 and S4 (Fig. 2), and vice versa at S1 and S2. This could be explained with the active denitrification zone being wider at S3 and S4, possible due to higher sedimentation rate or different sediment characteristics than at S1 and S2. However, the abundance pattern was similar in surface and deep samples, the abundance of *nirS* being highest at S1 and S3, and related neither to land-use nor water inorganic N concentrations (Spearman correlation,  $p < 0.05$ ). In contrast, the abundances of *nirK* and *nosZ* increased with nitrite + nitrate (*nirK*:  $\rho = 0.95$ ,  $p < 0.001$ , *nosZ*:  $\rho = 0.90$ ,  $p < 0.001$ ) and sediment organic matter (*nirK*:  $\rho = 0.71$ ,  $p = 0.001$ , *nosZ*:  $\rho = 0.78$ ,  $p < 0.001$ ), decreased with ammonium (*nirK*:  $\rho = -0.78$ ,  $p < 0.001$ , *nosZ*:  $\rho = -0.91$ ,

$p < 0.001$ ), and were related to land-use (field% vs. *nirK*:  $\rho = 0.96$ ,  $p < 0.001$ , *nosZ*:  $\rho = 0.93$ ,  $p < 0.001$ ), peaking at the agriculture-dominated S2 (Suppl. Figure 2). These results suggest that *nirS* and *nirK* exhibited some niche separation, since *nirK* carrying denitrifiers were more sensitive to environmental variation, as has been previously observed in soil environments (e.g. Enwall et al. 2010). In addition, the results indicate that genetic denitrification potential increased with higher availability of substrates and electron donors, the denitrification gene abundance being highest at the agriculture-dominated sampling point with high nitrite + nitrate and sediment organic matter availability.

To further estimate the genetic potential for  $N_2$  production, we examined the relationship between nitrite reduction genes (*nirS* + *nirK*) and nitrous oxide reduction gene (*nosZ*). We acknowledge that our primer pair could not amplify *nosZ<sub>II</sub>* clade (Sanford et al. 2012; Jones et al. 2013), and thus we captured only a part of *nosZ* population. However, the ratio between *nirS* + *nirK* and *nosZ* has indeed been found to be the one to govern  $N_2O$  reduction potential in boreal lakes, an increase in the ratio indicating higher genetic potential for  $N_2O$  than for  $N_2$  production, and a decrease in the ratio indicating higher  $N_2$  production potential (Saarenheimo et al. 2015a). The *nirS* + *nirK*/*nosZ* ratio followed land-use patterns by increasing with higher proportion of peatlands and ammonium (peatland%:  $\rho = 0.89$ ,  $p < 0.001$ , ammonium:



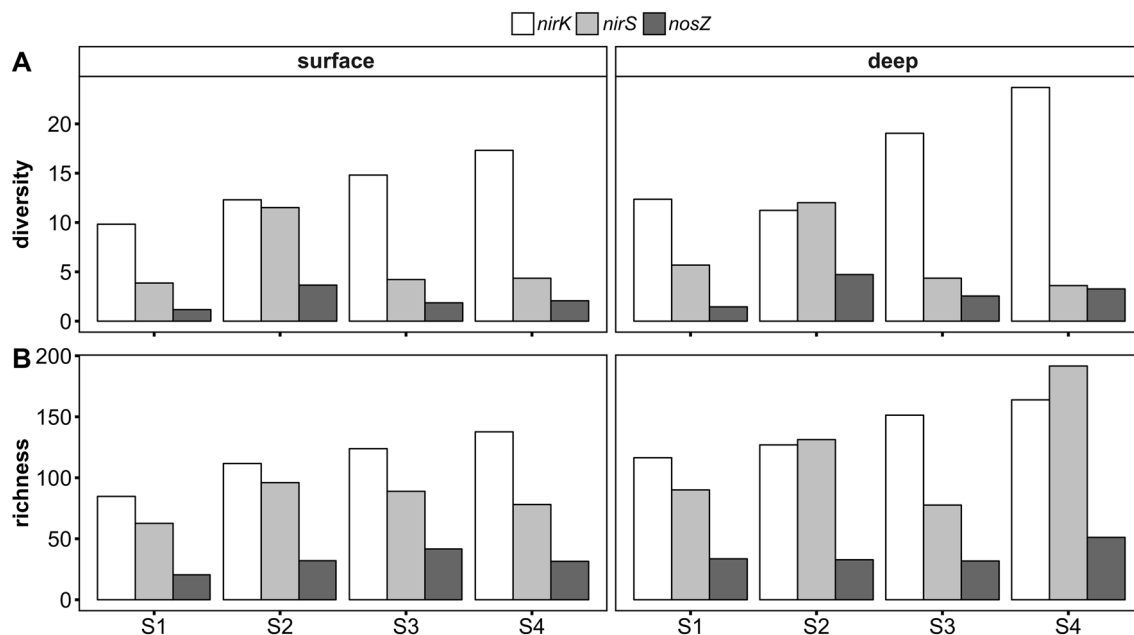
**Fig. 2** The abundances of *nirK*, *nirS* and *nosZ* genes in deep and surface sediment samples at four sampling points. The number below bars denote the ratio between *nirK* + *nirS* and *nosZ*

$\rho = 0.89$ ,  $p < 0.001$ ), and decreasing with higher proportion of fields and nitrite + nitrate (field%:  $\rho = -0.81$ ,  $p < 0.001$ , nitrite + nitrate:  $\rho = -0.72$ ,  $p < 0.001$ ), as *nirS* abundance remained high while the abundance of *nosZ* decreased at S3 and S4 (Suppl. Figure 2). The ratio was also negatively correlated with LOI% ( $\rho = -0.98$ ,  $p < 0.001$ ), %C ( $\rho = -0.75$ ,  $p < 0.001$ ), and D15 ( $\rho = -0.69$ ,  $p = 0.001$ ), indicating that carbon availability could limit the last step of denitrification and  $N_2$  production potential through affecting the denitrification gene abundances (Suppl. Figure 2). This finding is in agreement with the previous studies from soil environment (Attard et al. 2011; Petersen et al. 2012), where denitrification potential increased with increasing organic carbon, and was directly linked to *nosZ* gene abundance. Furthermore, the importance of carbon in controlling the end product of denitrification is supported by connection between higher carbon availability (higher ecosystem respiration) and decreased  $N_2O$  yield from denitrification in streams (Beaulieu et al. 2011).

### Denitrifying microbial communities

Denitrifier communities exhibited different diversity and richness patterns. The diversity and richness of *nirK* community increased from S1 to S4 both in deep and surface sediment samples, while in the *nirS* and *nosZ* communities, the diversity peaked at S2, and species richness increased at S2 and S4 in deep sediment samples (Fig. 3). The *nosZ* diversity and species richness were always lower than that of the other communities due to lower sequencing depth

(Fig. 3). The correlation analysis indicated that the *nirK* diversity increased with the higher proportion of peatlands and with increasing ammonium concentrations (peatland%:  $\rho = 0.75$ ,  $p < 0.001$ , ammonium:  $\rho = 0.74$ ,  $p < 0.001$ ), and decreased with increasing nitrate concentration ( $\rho = -0.62$ ,  $p = 0.007$ ). The *nirK* species richness behaved similarly to *nirK* diversity, indicating that prevailing environmental conditions support *nirK* carrying community throughout Löytynoja, and that *nirK* OTUs could accumulate to S4 collecting organic material from the upper stream parts. However, *nirK* communities exhibited lower diversity at nitrate-rich S2, which could be explained with some *nirK* community members being DNRA microbes (Helen et al. 2016), which are commonly promoted in environments with lower nitrate concentrations and high organic C loading (Hardison et al. 2015), and would thus be suppressed in nitrate-rich conditions, decreasing the overall *nirK* community diversity. The diversities of both *nirS* and *nosZ* communities were connected to land-use (field% vs. *nirS*:  $\rho = 0.97$ ,  $p < 0.001$ , *nosZ*:  $\rho = 0.45$ ,  $p = 0.020$ ) as well as sediment quality (LOI vs. *nirK*:  $\rho = 0.84$ ,  $p < 0.001$ , *nosZ*:  $\rho = 0.59$ ,  $p = 0.002$ ; %C vs. *nirK*:  $\rho = 0.58$ ,  $p = 0.002$ , *nosZ*:  $\rho = 0.91$ ,  $p < 0.001$ ; %N vs. *nirK*:  $\rho = 0.74$ ,  $p < 0.001$ , *nosZ*:  $\rho = 0.86$ ,  $p < 0.001$ ; Suppl. Figure 2), and *nirS* diversity increased with D15 ( $\rho = 0.44$ ,  $p = 0.026$ ), agreeing with the previous results on long-term N fertilization increasing both *nirS* and *nosZ* diversity (Bowen et al. 2013; Kearns et al. 2015) and denitrification rates (Peng et al. 2016) in salt marsh sediments. The species richness of *nirS* correlated with the sediment quality (%C:  $\rho = 0.74$ ,  $p < 0.001$ , %N:  $\rho = 0.56$ ,  $p = 0.003$ ), and *nosZ*

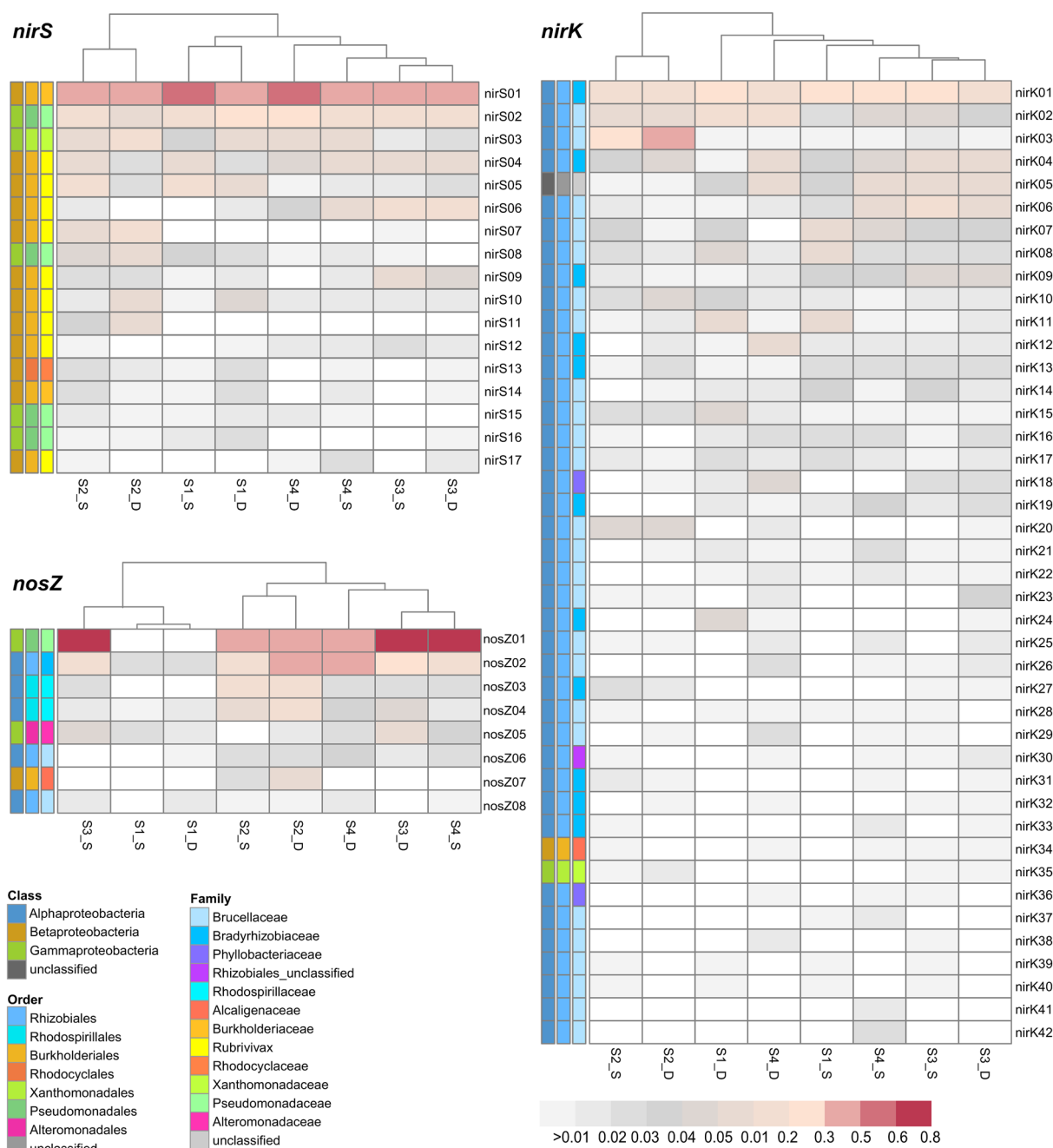


**Fig. 3** **a** The diversity, and **b** species richness of *nirK*, *nirS* and *nosZ* communities in deep and surface sediment samples at four sampling points

species richness was positively correlated with peatland proportion ( $\rho = 0.74$ ,  $p < 0.001$ ) and related environmental factors (DOC:  $\rho = 0.86$ ,  $p < 0.001$ ,  $\text{NH}_4^+$ :  $\rho = 0.73$ ,  $p < 0.001$ ; Suppl. Figure 2). For both *nirS* and *nosZ*, the high species richness and low diversity at S4 could indicate that OTUs could accumulate there from the other sampling points or come with the catchment loading, but the habitat characteristics still only support the abundance of certain OTUs. At

S2, the high *nirS* and *nosZ* diversity and species richness suggests that N-rich loading coming from catchment with agriculture-dominated land-use type supports novel denitrifiers by providing more niche space (Bowen et al. 2013).

The *nirK* and *nirS* communities clustered with land-use, S2 being separated from the other three sampling points, especially from the peatland-dominated S3 (Fig. 4). In *nirK* community, OTU1 (alphaproteobacterial



**Fig. 4** Heatmaps presenting the relative abundance of the core OTUs (representing 80% of total sequences) and UPGMA clusters of sampling point microbial communities in *nirS*, *nosZ*, and *nirK* communities in deep and surface sediment samples at four sampling points



*Bradyrhizobiaceae*; 13–30% of sequences in samples) was equally abundant at all sampling points, while OTU2 and OTU3 (alphaproteobacterial *Brucellaceae*) were more abundant at S2, and in the pooled S1 sample than in the five other samples. In the *nirS* community, the abundances of OTU1 (betaproteobacterial *Burkholderiaceae*) and OTU2 (gammaproteobacterial *Pseudomonadaceae*), comprising 41–78% of sequences in samples, were lower at S2 than at the other sampling points. Furthermore, OTU3 (gammaproteobacterial *Xanthomonadaceae*; 2–14% sequences) was more abundant at both S1 and S2 than the other two sampling points. Interestingly, OTUs representing betaproteobacterial *Rubrivivax* (OTU4–7, 9–12, 17) did not show a uniform pattern between the sampling points. The response of *nosZ* community was more diverse than of nitrite reducing communities, as samples from the two denitrification hotspot sampling points (S2 and pooled sample from S4) were grouped together. This was mainly explained with lower abundance of OTU1 (gammaproteobacterial *Pseudomonadaceae*) and higher abundances of OTUs 2–4 (alphaproteobacterial *Bradyrhizobiaceae* and *Rhodospirillaceae*) at these sampling points. When inspecting the co-abundance patterns between *nos* and *nir* communities (data not shown), we observed that the relative abundance of sequences related to OTUs possessing only *nosZ* was higher at S2 (27% of *nosZ* seqs) than at other sampling points (3–12% of *nosZ* seqs). The abundance of *nosZ* OTUs having *nirK* increased in S2 and pooled S4 samples (25–45% of *nosZ* seqs), while the relative abundance of *nosZ* OTUs having also *nirS* was high in all other samples (48–93% of *nosZ* seqs). Altogether, our results suggest that certain key taxa, possessing only *nosZ* or both *nosZ* and *nirK*, were favoured by land-use-driven N amendment and higher organic matter availability, and could explain the higher denitrification potential at S2 and S4.

### The effect of land-use on stream denitrification

Land-use can shape nitrate removal in the receiving streams and lakes, as has been suggested in the previous studies focusing either on microbial analysis (Bruesewitz et al. 2011) or evaluation of the denitrification potential (Arango et al. 2007; Eberhard et al. 2018; Inwood et al. 2005, 2007). Here, we combined the information on the abundance and community composition of denitrifying microbes to the isotope-based potential denitrification rate measurements for a more thorough evaluation on the effect of land-use on nitrate removal in a boreal stream. We demonstrated that the high substrate (nitrate) and carbon availability at the agriculture-dominated sampling point (S2) increased the genetic denitrification potential (denitrification gene abundance) and the abundance of certain key taxa and the overall diversity of denitrifying microbial communities. Concomitantly, we found high potential denitrification rates at that

sampling point, indicating that catchment land-use can affect N removal potential through altering abundance but also community composition of denitrifying bacteria, agreeing with previous findings from salt marsh sediments (Angell et al. 2018). However, high potential denitrification rates were also found at the lowest sampling point with peatland-dominated catchment area, but with high accumulation of organic matter originating from the whole upper catchment area. In conclusion, our results suggest that in addition to the catchment land-use, organic matter is the key factor in controlling N removal in Löytynoja, and possibly in other boreal streams receiving high N loading. Furthermore, as both community diversity and abundance of *nosZ* increased in sediments receiving high organic matter and N loading, our results imply that catchment land-use and sediment organic matter could especially regulate N<sub>2</sub>O yield from denitrification.

We acknowledge that our data are limited due to low number of data points taken only in one stream, so further studies on the land-use driven changes in nitrogen transforming microbial communities are needed for corroborating these findings. Nevertheless, our results suggest that in the future, when higher catchment loading and browning of aquatic ecosystems is expected (Kritzberg et al. 2014), the land-use type, together with water velocity affecting organic matter accumulation, could define N removal capacity in streams, as well as control whether they become N<sub>2</sub>O sinks or sources through altering denitrifier abundance and community composition.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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